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Phenotypic Plasticity and Contemporary Evolution in Introduced Populations: Evidence from Translocated Populations of White Sands Pupfish (Cyrpinodon tularosa)

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Keywords
Cyrprinodon, Evolutionary divergence, Morphological, Phenotypic plasticity, Pupfish

Disciplines
Ecology and Evolutionary Biology | Forest Sciences | Zoology

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SPECIAL ISSUE

Evolution in biological invasion

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Keywords Cyrprinodon · Evolutionary divergence · Morphological · Phenotypic plasticity · Pupfish

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Introduction

Phenotypic plasticity has traditionally been considered key to the success of invasive species colonizing new areas (Losos et al. 2000; Claridge and Franklin 2002; Dybdahl and Kane 2005; Spector and Putz 2006). Because invasive species typically exist at low numbers when they first colonize a new area (Schmitz et al. 1997), phenotypic plasticity is a mechanism by which small populations are potentially buffered against strong selection. However, mounting evidence (e.g., Huey et al. 2000; Maron et al. 2004) suggests that rapid genetically based adaptations to novel environments are quite important in the ecology of invasions. The most recent studies attempt to distinguish between phenotypic plasticity and genetically based adaptation rather than consider whether phenotypic plasticity is concordant with contemporary evolution (e.g., Dybdahl and Kane 2005; but see Losos et al. 2001). This emphasis is perhaps entrenched in the assumption that phenotypic plasticity produces a wide range of phenotypes and precludes selection from acting on traits, thereby retarding directional evolution (West-Eberhard 2003). However, phenotypic plasticity may also accelerate directional evolution by producing recurrent expression of environmentally matched phenotypes. The extent to which phenotypic plasticity promotes or retards directional evolution of newly established populations is perhaps context-dependent and related to the relative amount of

phenotypic variation due to genetic variation. Thus, experimental evidence on the breadth of phenotypic plasticity may indicate the direction and tempo of evolution for invasive populations in novel environments.

Much can be learned about the contemporary evolution of invasive species from studies of conserved species, where populations are often replicated in novel environments as a hedge against local extinction (Stockwell and Weeks 1999; Stockwell and Leberg 2002; Stockwell et al. 2003). Pupfishes of North American deserts provide good model systems for such studies because many pupfish conservation plans contain a component for population replication (Deacon and Deacon-Williams 1991; Stockwell and Weeks 1999). Additionally, pupfishes can tolerate a broad range of environmental conditions in desert aquatic habitats, including extremes in temperature and salinity (Miller 1948, 1981), suggesting that phenotypic plasticity is a valuable attribute of their ability to colonize disparate habitats.

Earlier morphological work on the White Sands pupfish (*Cyprinodon tularosa*) showed that native populations exhibit both genetically based body shape divergence and phenotypic plasticity in body shape in response to varied salinity levels (Collyer 2003; Collyer et al. 2005). This desert fish species has both historic and recently introduced populations occurring in ecologically disparate environments. Specifically, a native population of White Sands pupfish, which naturally occurs in a highly saline, riverine environment, was introduced to brackish, lacustrine environments at different times in the recent past (three decades and 1 year prior to this study) (Stockwell et al. 1998; Pittenger and Springer 1999). In the current study, we evaluate morphological divergence of riverine White Sands pupfish associated with these introductions. Previous work has shown that the three-decade-old population manifests a greater morphological divergence from its source than that between native populations isolated several thousand years in similarly contrasting environments (Collyer et al. 2005). Contemporary evolution has been implicated for such divergence based on indirect experimental evidence of native pupfish raised in controlled saline mesocosms (Stockwell and Collyer, unpublished data).

The objective of this study was to determine if the morphological divergence of the approximately 30-year-old population could be achieved within 1 year after a similar ecological event (transfer of pupfish from saline river to brackish, lacustrine habitats). If shape differences after 30 years since colonization can be attributed to phenotypic plasticity, we hypothesize that there should be no evidence of distinction of the 30-year-old population divergence relative to the 1-year-old populations (i.e., the shape difference between the 30-year-old population and its source population would be no different than that for the 1-year-old populations and the same source population, if the shape difference is due solely to phenotypic plasticity). Evidence supporting this hypothesis would suggest that phenotypic plasticity in response to salinity is so great that it retards directional evolution. Alternatively, evidence of a distinction of the 30-year-old population would provide support for contemporary evolution, especially if the directions of shape change are consistent among introduced populations (i.e., parallel shifts due to consistent responses to similar selection; see also Losos et al. 2000, 2001).

Study system

The White Sands pupfish is designated as a threatened species by the state of New Mexico. One native population occurs at Salt Creek (SC), where it has been presumably isolated since the desiccation of the Pleistocene Lake Otero, approximately 5,000–7,000 years ago (Miller and Echelle 1975; Pittenger and Springer 1999). Salt Creek was the largest tributary of Lake Otero and currently has a perennial stream reach of approximately 30 km from a maximum elevation of about 1250 m (Pittenger and Springer 1999). The incised channel of this endorheic stream is 2–4 m deep throughout its range. (See Pittenger and Springer 1999 for more details.) Salt Creek is highly saline (11–40 ppt), although salinity and temperature (3.0–33.4°C) can be quite variable in this habitat due to variable water flow (Stockwell and Mulvey 1998).

Mound Spring (MO) is located approximately 7 km northwest of Salt Springs, the source of Salt Creek, and at an elevation of 1323 m, it never has had a perennial connection to Salt Creek or Lake Otero. Historically, Mound Spring was a small seep, but it was excavated by dragline in 1967, producing a pond with a surface area of approximately 400 m^2 (Pittenger and Springer 1999). The maximum depth of the pond is reported to be 3.4 m at the spring, but the depth of the pond is less than 1 m in most places. Pupfish were observed in the pond in 1973 and presumed to have been introduced around 1970. Although the introduction was not documented, the founding number of pupfish is assumed to be small. Allozyme and microsatellite DNA analyses were used to identify the source of this population as SC pupfish (Stockwell et al. 1998). Mound Spring is brackish (1.5–4.0 ppt) and less variable in temperature (9.4–26.3°C; Stockwell and Mulvey 1998) than Salt Creek. Thus, the contrast in environments between the saline creek and brackish spring, plus the relationship between streamlining and salinity (Videler 1993; Webb 1997), set the stage for potential adaptive divergence at Mound Spring.

A previous study indicated that riverine SC pupfish are characterized by more slender body shapes than the lacustrine MO pupfish (Collyer et al. 2005). Drag reduction via streamlining because of greater water flow and higher salinity (i.e., greater density and viscosity) in the riverine environments was identified as a putative mechanism leading to shape differences among populations. The genetic basis for body shape was confirmed

from laboratory cultures of White Sands pupfish (Stockwell and Collyer, unpublished data). These data thus suggested a case of contemporary evolution in body shape between slender bodied and deep-bodied forms in the native Salt Creek and non-native Mound Spring populations, respectively.

In the spring of 2001, 15 prototype refuge populations were established in artificial ponds (as part of a long-term evolutionary and ecological study on translocations of White Sands pupfish populations as a conservation strategy). Nine of these populations were derived from SC pupfish. Each artificial pond consisted of an earthen rectangular pit, with surface dimensions of approximately 8 × 12 m, located at Holloman Air Force Base, New Mexico. The ponds were staggered on either side of a main pipe, which supplied water (tertiary treated sewage effluent with chlorine removed) to the ponds. Each pond was routinely filled to a depth of approximately 1 m from a spigot from the main pip, situated approximately 1.5–2.0 m above the deepest portion of the pond. The ponds were initially filled in March 2001 to allow aquatic invertebrates and vegetation to become established before pupfish were introduced in late May and early June. Each "pond" population was founded by 100 female and 100 male young-of-the-year pupfish collected from Salt Creek.

White Sands pupfish mature and breed readily in their first year; thus, these populations were represented mostly by non-founders the following year (Table 1). In May 2002, seven pond populations created from SC fish existed. Six of the seven SC-stocked pond populations were used in the current study because two populations had gone extinct and another population had very low fish densities, which precluded the collection of fish.

Salinity and temperature of the refuge ponds closely mimicked the Mound Spring environment in the first year of their existence (Table 1). Thus, establishing pond populations with SC pupfish mimicked the creation of the MO population three decades prior, in terms of confrontation of a new environment defined by salinity and temperature (other abiotic and biotic factors notwithstanding).

Methods

A total of 417 individuals (203 males and 214 females) from a 2002 voucher collection of White Sands pupfish were used in this study. These 2002 vouchers came from eight populations: two wild populations (MO and SC) and six populations from the artificially created ponds (P-8, P-9, P-11, P-14, P-17, P-18). The 417 (out of 500 total) pupfish vouchers were a subset of 1-year-old adults collected. Using only 1-year-old fish reduced the potential for "year" effects because older fish were present in the collection from the two wild populations. Pupfish were collected with a combination of minnow traps and beach seines; SC pupfish were collected from the middle section of Salt Creek (described in Stockwell and Mulvey 1998).

To quantify body size and shape we used landmark-based geometric morphometric methods (Rohlf and Marcus 1993; Adams et al. 2004). First, digital photographs were collected on anesthetized live fish, using an Olympus Powershot G1 digital camera, shortly after the fish were collected. The x, y Cartesian coordinates of 13 anatomical landmarks (Fig. 1) were recorded from each image and used to estimate size and generate a set of shape variables that were independent of non-shape variation (i.e., effects of specimen size, orientation, and position were held constant). Specimen centroid size

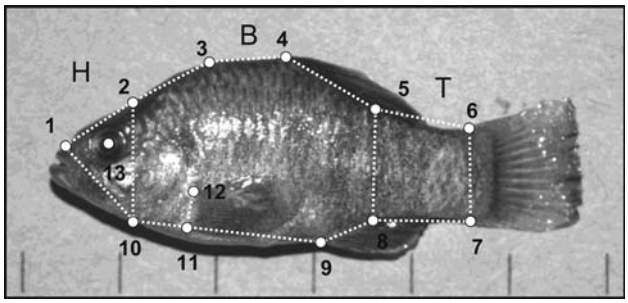

Fig. 1 Representative male White Sands pupfish (from Mound Spring) and illustration of the 13 landmarks used for morphometric analyses. The head (*H*), body (*B*), and tail (*T*) regions are emphasized to facilitate visual understanding of shape differences

Table 1 Minimum, mean, and maximum temperature, salinity, specific conductivity, and estimated population size for artificial ponds of White Sands pupfish

Pond	Temperature (°C)			Salinity (‰)			Specific conductivity (mS)			n[a]		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	95% LCL	Mean	95% UCL
8	4.9	15.8	28.8	1.6	3.5	4.6	3.8	6.3	8.3	1994	2153	2344
9	7.1	16.5	28.0	2.1	3.3	3.5	3.1	5.9	8.5	455	461	470
11	5.6	16.6	28.4	1.6	2.3	3.8	3.1	4.1	5.4	336	342	350
14	5.5	16.5	28.1	1.5	2.5	3.8	3.1	4.6	7.0	888	924	970
17	4.7	16.0	30.0	2.2	3.3	9.6	3.3	6.0	16.5	680	692	707
18	4.7	15.8	29.0	2.0	3.5	8.4	3.9	6.3	14.4	762	766	773

Data for ponds are summarized from 40 field measurements made between June 2001 and May 2002 (Collyer 2003)

[a] Lincoln-Peterson population size estimates (n) with 95% confidence limits (CL; U, upper; L, lower) are from a mark-recapture study performed in August 2002 (Stockwell, unpublished data)

(CS, the square root of summed square distances of landmarks from the configuration centroid; Bookstein 1991) was measured for the total landmark configuration (Fig. 1). CS was also measured separately for head, body, and tail regions because differences in relative size of body proportions can influence geometric shape (Weaver 2003), possibly confounding biological interpretations. Shape variables were generated from a generalized Procrustes analysis (Rohlf and Slice 1990), followed by a thin-plate spline analysis (Bookstein 1991) for the full set of landmarks. Shape variables generated by this procedure can be used to test hypotheses about shape with standard multivariate statistical procedures (see e.g., Adams and Rohlf 2000; Adams 2004; Collyer et al. 2005; Adams et al. 2007).

Analysis of variance (ANOVA) was performed on total CS to test for differences among populations, and analysis of covariance (ANCOVA) was performed on head, body, and tail CS to test for differences among populations, holding the effect of overall specimen size (total CS) constant (i.e., relative head, body, and tails sizes were compared among populations at a common overall size). To assess shape variation between populations, and with respect to allometry (shape covariation with size), we compared three models of shape variation. The first included parameters to estimate population means in shape irrespective of specimen size (POP). The second model also included total CS as a covariate to account for variation in shape due to shape allometry with respect to overall specimen size (POP + CS_{Total}). The third included head, body, and tail CS as covariates, rather than total CS, to account for variation in shape due to differential allometries (POP + CS_H + CS_B + CS_T). These models were evaluated with multivariate analysis of variance (MANOVA) and compared using a multivariate generalization of Akaike's information criterion (AIC; Bedrick and Tsai 1994).

The shape difference among pairs of populations was quantified as the Euclidean distance (D) between population means, accounting for shape allometry with the most appropriate shape model. To assess the significance of observed Euclidean distances, we performed a permutation procedure (sensu Collyer and Adams 2007; Adams and Collyer 2007) that preserves the overall shape allometry effects. This procedure involves estimating parameters for size and population effects, removing the population effect and randomizing residuals (thus, preserving the shape/size relationship), and re-estimating population parameters from randomly assigned residuals. This procedure was performed 9999 times to generate distributions of 10,000 random pairwise distances (including observed values) between populations. The proportion of randomly generated distances (of 10,000) greater than or equal to the observed distance was treated as the significance level of the observed value (see, for example, Adams and Rohlf 2000; Klingenberg and Leamy 2001; Collyer and Adams 2007).

To visualize shape variation among populations, we performed a principal components analyses (PCA) on shape residuals from the multivariate multiple regression of shape involving CS_H, CS_B , and CS_T. Plots were generated for the first two principal components (PCs) of this variation, which describes the relative differences in shape of each population independent of variation due to shape allometry. Graphical deformations of the male and female mean landmark configurations, superimposed on predicted population-specific configurations, were performed to visualize shape differences analogous to D'arcy Thompson's (1917) transformation grids. All analyses were performed with the TPS series of software (Rohlf 2004), NTSYS-pc ver. 2.1 (Rohlf 2002), JMP ver. 5.1 (SAS Institute 2002), and Poptools ver. 2.6.6 (Hood 2005). Sequential Bonferroni adjustments (Rice 1989) of the Type I error rate (α) were applied for analyses that involved pairwise comparisons (i.e., Euclidean distances among populations) to maintain an overall α equal to 0.05.

Results

Significant variation in head, body, tail, and total size was found among populations of White Sands pupfish (Tables 2,3; Fig. 2). Wild populations (MO and SC) had similar average head and total individual sizes, but they differed significantly in body and tail sizes, with MO pupfish having proportionately smaller bodies and larger tails than their progenitors at SC. Pupfish from

Table 2 Results of analyses of variance (ANOVA) for centroid size (CS) measures showing specific contrasts for male pupfish

Response	Population		CS_{Total}		Contrast P-SC		Contrast P-MO		Contrast SC-MO	
	$F_{7,194}$	P	$F_{1,194}$	P	$F_{1,194}$	P	$F_{1,194}$	P	$F_{1,194}$	P
CS_H	21.749	< 0.001	1,132.65	< 0.001	93.754	< 0.001	92.817	< 0.001	9.296	0.002
CS_B	7.648	< 0.001	22,889.01	< 0.001	28.341	< 0.001	1.864	0.174	40.951	< 0.001
CS_T	2.869	0.007	1,640.225	< 0.001	17.542	< 0.001	4.931	0.028	7.284	0.007
CS_{Total}	20.403	< 0.001	–	–	55.142	< 0.001	80.808	< 0.001	1.787	0.183

Head (H), body (B), and tail (T) measures of CS were tested for differences among populations, with total CS used as a covariate, to account for the variation in these values associated with overall pupfish size. Contrasts involved comparisons between pond (P) populations and both Salt Creek (SC) and Mound Spring (MO) populations, and between MO and SC populations

Table 3 Results of ANOVA for CS measures with specific contrasts for female pupfish[a]

Response	Population		CS_{Total}		Contrast P-SC		Contrast P-MO		Contrast SC-MO	
	$F_{7,205}$	P	$F_{1,205}$	P	$F_{1,205}$	P	$F_{1,205}$	P	$F_{1,205}$	P
CS_H	20.980	< 0.001	905.300	< 0.001	70.005	< 0.001	111.938	< 0.001	0.001	0.990
CS_B	13.842	< 0.001	20,844.062	< 0.001	6.754	0.010	47.679	< 0.001	68.599	< 0.001
CS_T	11.394	< 0.001	1,121.607	< 0.001	30.359	< 0.001	9.424	0.002	66.612	< 0.001
CS_{Total}	29.888	< 0.001	–	–	76.793	< 0.001	119.421	< 0.001	0.399	0.528

[a] See footnotes of Table 2 for explanation of terms

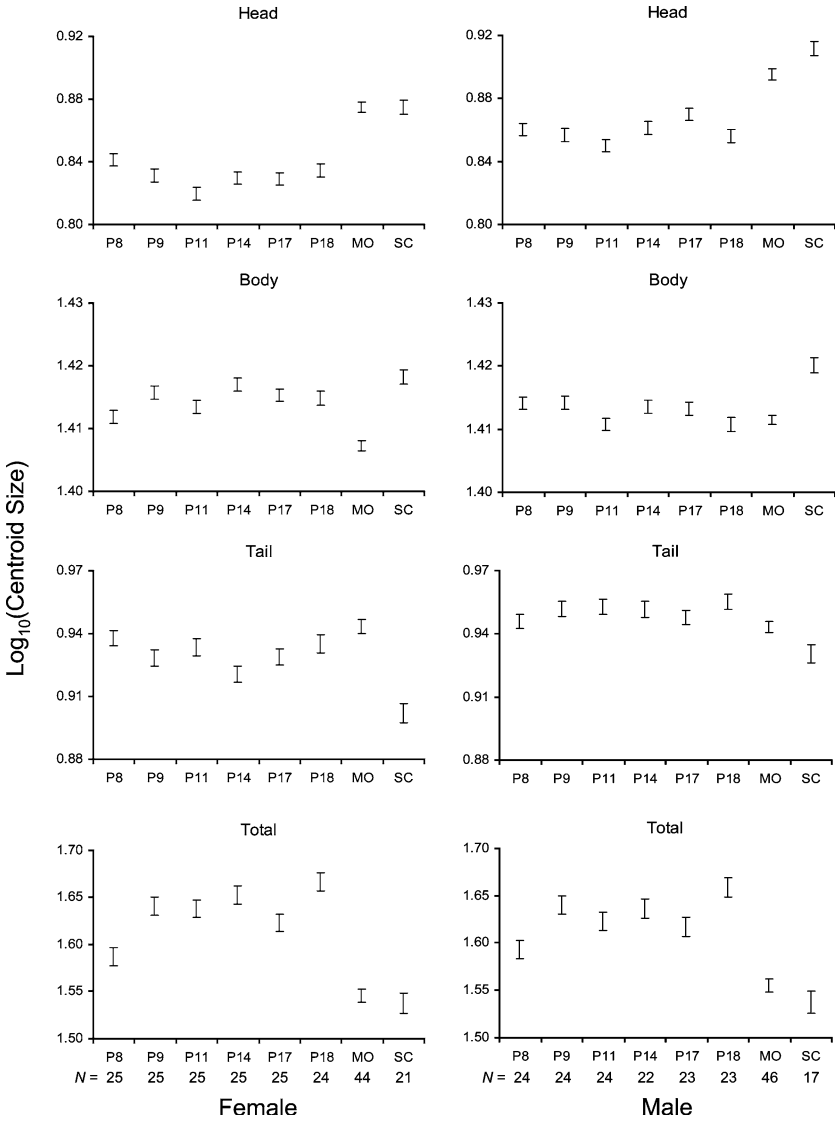

Fig. 2 Log_{10}(centroid size) plots for the head, body, tail, and total landmark configurations for female (*left*) and male (*right*) White Sands pupfish. Sample sizes are given *below* the population labels and *error bars* represent ± one standard error of the mean (SEM)

the artificially created ponds were more or less similar in all size measures, with the exception of the P-8 population which had a smaller average total size their counterparts in the other ponds. Although wild populations were significantly smaller in terms of average individual size than pond pupfish, both the MO and SC populations had relatively larger heads (Fig. 2). The body size of MO male pupfish was similar to that of male pond pupfish, while the body size of female MO pupfish was significantly smaller than that of female pond pupfish, whereas the body size of SC pupfish was significantly larger than that of pond pupfish for both males and females. Tail sizes were intermediate for pond pupfish: significantly larger than SC pupfish, but significantly smaller than MO pupfish. Taken together, these results indicate a differential growth of body

Table 4 Multivariate analysis of variance statistics (MANOVA) and model comparison values (ΔAIC) for three different models of shape variation

Models	Wilks Λ	Estimated F	df_{Model}	df_{Error}	P	ΔAIC
Male						
Population	0.0263389	5.508	154	1177.166	< 0.0001	2412.292
Pop. + CS_{Total}	0.0053559	7.498	176	1321.522	< 0.0001	2220.942
Pop. + CS_H + CS_B + CS_T	0.0000001	33.121	220	1586.936	< 0.0001	0
Female						
Population	0.0211215	6.321	154	1250.882	< 0.0001	2360.831
Pop. + CS_{Total}	0.0087303	6.970	176	1404.598	< 0.0001	2303.763
Pop. + CS_H + CS_B + CS_T	0.0000003	32.315	220	1687.503	< 0.0001	0

Analyses were performed on males and females separately

regions between pond pupfish and both MO and SC pupfish.

Differences in body region growth among wild and pond populations are also likely to impact how shape allometries should be defined, a conclusion justified from a comparison of shape models (Table 4). Models including separate measures of head, body, and tail size explained impressively more shape variation than models including only a single overall measure of size or excluding size altogether. The projection of average population effects from this model onto the first two PCs of residual shape variation demonstrated that pond pupfish shapes tended to be intermediate between SC and MO (Fig. 3). MO and SC populations differed significantly from one another (males: $D = 0.023$, $P_{RAND} = 0.0003$; females: $D = 0.029$, all $P_{RAND} = 0.0001$), and these shape differences were the largest among all population comparisons (Table 5). Significant differences between pond pupfish shapes and either SC or MO shapes (Table 5) were observed for only seven of the possible 24 comparisons (six pond populations × two wild populations × two sexes). SC pupfish had the most dorsally flattened body shapes and MO pupfish had the most deep-bodied shapes among all populations, although all pond shapes exhibited some degree of body deepening. Pond pupfish had more dorsally directed head shapes (landmarks 1, 2, and 10) than either SC or MO pupfish.

Significant divergences from the SC shape were observed for three of the six pond populations – but only for females. In two of these cases (P-9 and P-14), shape did not significantly differ from the MO shape, indicating a convergence toward the MO form. P-18 females were significantly different in shape from MO females, but not from SC females, indicating retention of the SC shape for this population. Only P-8 females exhibited significant distinction from both SC and MO shapes, characterized by deeper bodies than the SC shape, but longer tails and more dorsally directed heads than either the SC or MO shapes. P-8 males, however, were significantly different in shape than MO males, exhibiting a similar trend (but not to the extent that they significantly differed from SC males). P-14 males were also significantly different than MO males, but not SC males, having a similar shape to P-8 males. Within pond populations, only the P-8 population was significantly different than any other pond population (three significant

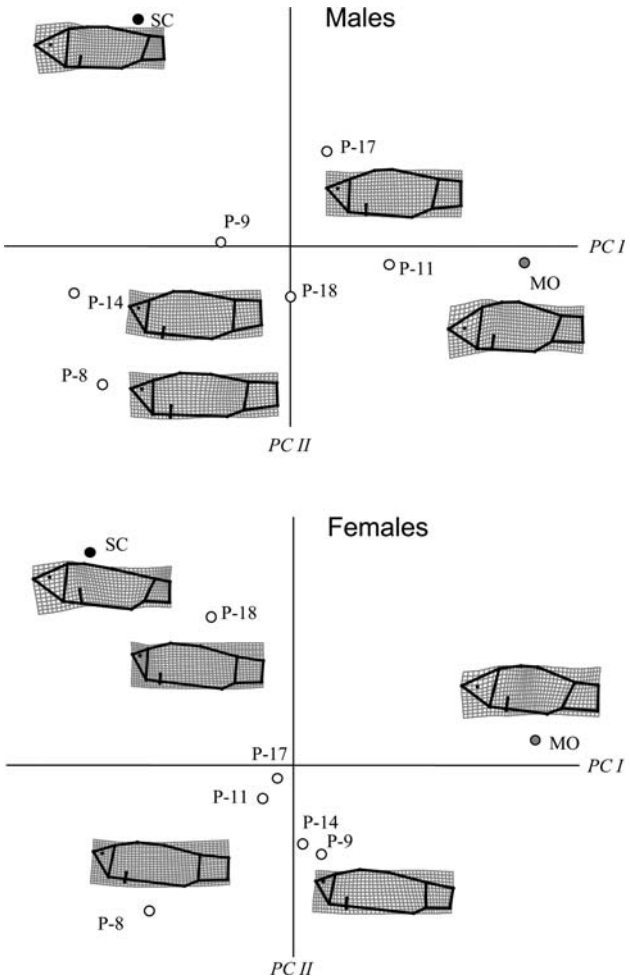

Fig. 3 Principal component plots of shape variation for White Sands pupfish (*top panel* for males, *bottom panel* for females). Principal components (PCs) were calculated for shape residuals after regressing shape against head, body, and tail log (CS). Values in plots correspond to population means projected onto the first two PCs. Deformation grids correspond to the deformation of the overall mean form for each population effect. These plots correspond to 41.9 and 47.2% of the residual variation for males and females, respectively

Table 5 Euclidian distances between least squares means from the MANOVA on shape for populations of White Sands pupfish

	P8	P9	P11	P14	P17	P18	MO	SC
P8		0.016	**0.022**	**0.022**	**0.022**	0.017	**0.023**	0.019
P9	0.017		0.016	0.013	0.013	0.016	0.017	0.014
P11	0.013	0.012		0.017	0.009	0.009	0.011	0.019
P14	0.015	0.010	0.013		0.013	0.018	**0.023**	0.018
P17	0.015	0.011	0.012	0.011		0.013	0.015	0.014
P18	0.016	0.018	0.014	0.018	0.013		0.013	0.017
MO	**0.025**	0.016	0.017	0.018	0.017	**0.021**		**0.023**
SC	**0.022**	**0.024**	0.018	**0.022**	0.020	0.015	**0.029**	

Values above diagonal are for males, values below are for females. All significant values (experiment-wise $\alpha = 0.05$) from permutation tests are in bold type

differences), and then only for males. These significant differences appear to be related to longer tail segments and slightly shallower body segments.

Discussion

Much knowledge can be gained by investigating character divergence of experimentally introduced populations (Simberloff et al. 2000). Using North American pupfishes as model systems is especially useful because the time of population introductions is typically known. Further, inference about the pace of phenotypic change can be gained from studies involving populations that experienced environmental change at different time periods. White Sands pupfish from a saline creek were introduced to brackish environments three decades (Mound Spring) and 1 year (experimental ponds) prior to this study. We predicted that morphological divergences would be similar in direction and magnitude for all introduced populations – if the MO divergence was a result of phenotypic plasticity. We found evidence to suggest that MO shape divergence was greater than pond shape divergences, although the directions of shape divergence were rather consistent. However, phenotypic plasticity in body size was more difficult to evaluate because of differential growth in body regions.

By decomposing overall individual size into head, body, and tail components, we found that pond pupfish had relatively smaller heads than either of the two wild populations, similar or slightly smaller bodies than SC pupfish, and similar or slightly larger tails than MO pupfish. Thus, pond pupfish had body sizes that tended to remain similar to SC pupfish but achieved a greater size by increased tail growth in brackish lacustrine environments. We conclude that in terms of regional body size, the differences between SC and MO body sizes, characterized by smaller bodies and larger tails in the brackish lacustrine environment, are possibly a result of phenotypic plasticity. However, plastic responses were also unpredictable for size measures: pond pupfish had significantly smaller relative head sizes than both wild populations, indicating that factors other than salinity and water flow (e.g., different aquatic prey or different intraspecific competition for the same prey as a result of small initial population size) could promote accelerated posterior body growth.

Because shape and size are likely to be phenotypically integrated (Pigliucci 2003), it is not surprising that the shape model that accounted for different shape allometries was exceptionally better than models using one or no size parameters (Table 4). By regressing shape on the three different size measures and determining population-specific departures from common shape/size allometries, we found that pond pupfish tended to be intermediate in body shape between MO and SC forms. The shape difference between MO and SC pupfish was highlighted by the increased body depth and tail length in the brackish lacustrine environment (Mound Spring). Pond pupfish shapes tended to exhibit slight increases in body depth and tail length as well, although there was evidence of discordant shape changes among pond populations (e.g., P-8 pupfish had longer tails and more slender body shapes than some other pond populations). It is important to note that with few exceptions, the shape divergences of pond populations from the SC form were not significant, and in only one case (P-8 females) was a pond population significantly different in shape from both the SC and MO forms (i.e., populations that significantly diverged from SC tended to converge on the MO shape). Further, in no case was a pond population as divergent in shape from SC as the MO shape, for both males and females. We thus conclude that phenotypic plasticity in body shape is not likely to account for the magnitude of body shape divergence of the MO population, unless an environmental parameter (other than salinity or water flow) that differs markedly between MO and pond environments promotes body deepening.

If we assume that salinity and water flow influence streamlining in fishes (see, for example, Weihs and Webb 1983; Videler 1993; Vogel 1994; Webb 1997) and large contrasts in these environmental parameters between environments produces a large selection difference, then the patterns we observed provide insight for how streamlined pupfish populations respond to directional selection for body deepening. We might also assume that the responses of 1-year pond populations are a result of phenotypic plasticity (i.e., insufficient time for evolutionary divergence). As the shape divergences of these populations tended not to be significant, and when significant, they tended in the direction but not to the same extent of the MO divergence, it seems reasonable to conclude that the MO population has evolved in contemporary time. Alternatively, we might assume that selection was strong enough to act on introduced populations in one generation (i.e., that we really did not measure divergence due to phenotypic plasticity, but rather evolutionary divergence after one generation) or that shape divergences of 1-year-old populations were due to a combination of phenotypic plasticity and evolutionary divergence. It remains unlikely that phenotypic plasticity retards directional evolution in this

system in response to selection for body deepening in brackish lacustrine environments. The magnitude of MO shape divergence was not reproduced, irrespective of the mechanism of shape change in pond populations.

Although the tendency of lacustrine pupfish to be deeper bodied than the SC form is consistent with a hypothesis that phenotypic plasticity may promote directional phenotypic evolution via phenotypic accommodation (adaptive phenotypic adjustment, without genetic change, following a novel input during development; West-Eberhard 2005), further experimental research is needed to isolate whether phenotypic plasticity speeds the rate of evolution. Our results indicate that shape divergence due to plasticity was more or less in the predictable directions, suggesting that directional evolution may be jump-started by phenotypic plasticity. However, there are certain aspects of our results that indicate it is difficult to gauge the course of evolutionary divergence. First, the P-8 population was the most distinct in shape among pond populations and least convergent on the MO form. It was also, exceptionally, the pond population with the greatest population density after 1 year (Table 1). Although unknown estimates of population size for MO and SC populations, plus the small number of ponds used in this study, precluded a meaningful test of shape and population density association, it appears that the P-8 shape was influenced by the exceedingly larger population size in this pond (P-8 pupfish were also smaller; Fig. 2). It is not clear from our data how population density might augment or retard the rate or direction of shape evolution, especially after several generations.

Second, MO pupfish were not introduced to the ponds because it was neither the intent of the original study (i.e., we obtained shape information from fish in these populations, but the populations were not created in order to obtain shape information) nor possible due to historical concerns about population sizes at Mound Spring by the regulatory agencies. However, a future experiment that includes a MO introduction would provide a control for discerning if there are body shape differences that result from environmental differences between Mound Spring and the artificial ponds. Nevertheless, this type of research would confirm the generality of body shape evolution in response to fluvial and salinity changes, but it would not change the fact that rapid phenotypic divergence is likely for refuge populations in this system.

The efficacy of population replication as a conservation strategy can be argued in the context of contemporary evolution. It has not escaped our attention that rapid phenotypic divergence for species of conservation concern, whether genetic or because of phenotypic plasticity, can be viewed as a bonus; such species might be less likely to go extinct because of environmental change. Although the goal for conserved species is to limit extinction risk – thus, an understanding of the evolutionary biology of introduced populations is essential – the knowledge gained from closely monitored colonization events also sheds light on the evolutionary biology of invasive species. In both cases, introduced or invading populations are typically small in number and experience novel environmental conditions. There is increasing awareness that phenotypic plasticity is not necessarily the sole mechanism by which invasive species can colonize heterogeneous environments (e.g., Dybdahl and Kane 2005). The results of our study are consistent with those of Dybdahl and Kane's (2005) study on invasive freshwater snails (*Potamopygrus antipodarum*) in that within decades, phenotypic differences of introduced populations exceeded expectation due to phenotypic plasticity, but phenotypic plasticity was intermediate in the same direction of evolved phenotypic change. This pattern might be pervasive for species that are successful invaders.

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